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A Preliminary Study of Healing of Superpulsed Carbon Dioxide Laser Incisions in the Hard Palate of Monkeys

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Background and Objective: **Prior studies of laser wound healing using different animal models have shown a delayed tissue response after carbon dioxide (CO_2) laser application. This article reports on the preliminary findings of healing of superpulsed CO_2 laser and scalpel incisions in the hard palate of monkeys.**
Study design/Materials and Methods: **Twelve parallel incisions using a superpulsed, continuous wave CO_2 laser and a scalpel were performed in the hard palate of each of two adult monkeys at 3, 7, and 14 days time schedules. Power levels of 2.0, 4.0, and 6.0 Watts were used for the laser incisions. Wounds were harvested, fixed in 10% formalsaline for at least 48 hours and processed routinely. Each specimen was embedded in paraffin wax at 90 degrees to the surface epithelium and 5 μm thick sections prepared for staining with haematoxylin and eosin, Periodic acid Schiff and Masson-trichrome at a step-serial interval of 100 μm. Sections were evaluated independently.**
Results: **According to the clinical findings we showed a wound closure in all of the wounds (laser and scalpel incisions) at 3, 7, and 14 days of healing. Histologically, we showed that laser incisions at three and seven days demonstrated an increased, power setting-dependent tissue necrosis and marked inflammatory response with minimal organization compared to scalpel incisions. At 14 days both types of incisions exhibited complete wound healing of the epithelium and connective tissue.**
Discussion and Conclusions: **According to these preliminary results, superpulsed CO_2 laser tends to produce more pronounced changes (due to tissue thermal damage) with corresponding greater inflammatory reaction and delay in tissue organization only initially. Lasers Surg. Med. 24:368–374, 1999.**

Key words: CO_2 laser; monkey; superpulse; oral tissue; wound healing

INTRODUCTION

The carbon dioxide (CO_2) laser is a unique energy system that is widely utilized as an effective tool in soft tissue surgery. With the CO_2 laser the major advantages are the production of local haemostasis thereby creating a bloodless surgical

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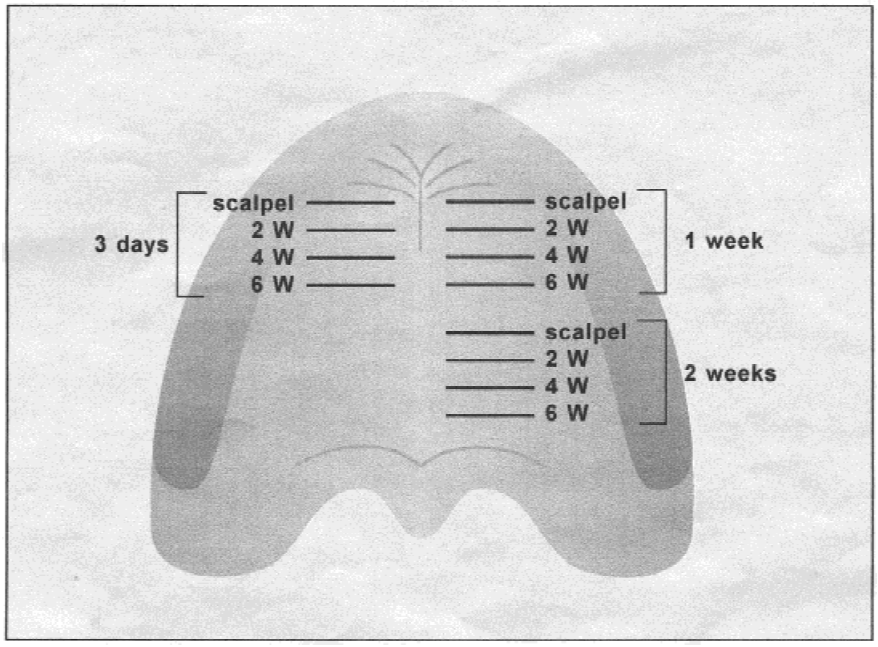

Fig. 1. Schematical drawing of the incisions performed in the hard palate.

field, bacterial elimination, and contact-free incision [1,2]. Postoperative pain is also notably minimized with laser incisions. The disadvantage of the laser is the induction of thermocoagulation and vacuolization tissue artefacts. To minimize this unwanted thermal damage, the use of superpulsed mode CO_2 laser based on the principles of high irradiation with short duration pulses and adequate pulse intervals has been advocated. Prior wound healing studies using the CO_2 laser showed a delayed healing in oral soft tissues [3,4] or after high power Nd:YAG laser application in the skin [5]. The aim of this study was to present clinical and histological wound healing characteristics in oral soft tissues after the application of a superpulsed CO_2 laser using the monkey as an animal model.

MATERIALS AND METHODS

Two adult monkeys of the species *Macaca fascicularis* were used for this study. The mucosa of the hard palate was selected for the oral mucous membrane wounds because of its accessibility. The animals were anaesthetized using Zoletil® (Verbac Laboratories, France). Two types of wounds were introduced with both stainless steel scalpel and a superpulsed, continuous wave mode CO_2 laser (Luxar, Boston, MA). The tissues were incised without any elevation of a full mucoperiosteal flap. Twelve parallel incisions (approx. 10–15 mm in length) were performed in the hard palate of each of these two monkeys at 3, 7, and 14 days time schedules (Fig. 1). The incisions were not sutured finally. Power output levels of 2.0, 4.0, and 6.0 watts were used for the laser incisions. The ceramic tip (Ø 0.8 mm) of the straight handpiece was in a distance of approximately 0.5 cm from the epithelial surface during laser expo-

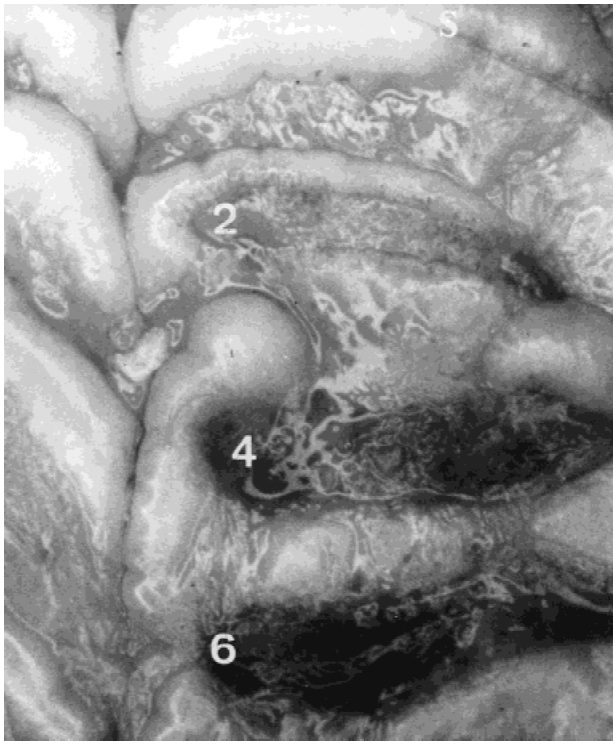

Fig. 2. Wound healing three days after surgery.

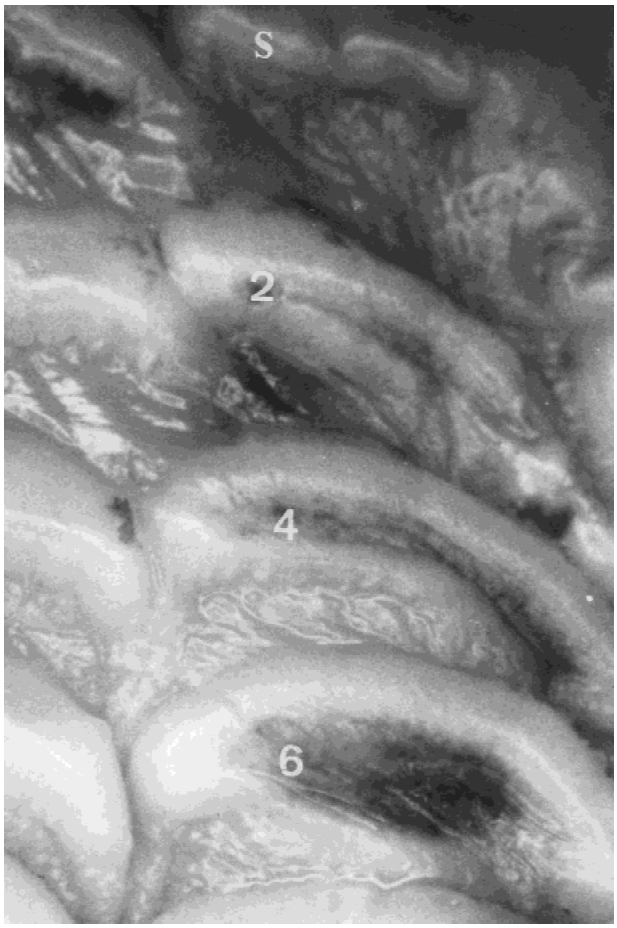

Fig. 3. Wound healing seven days after surgery.

Fig. 4. Wound healing 14 days after surgery.

sure (six seconds). At each sacrifice, clinical photographs were taken. Wounds were harvested with a margin of normal tissue and placed in 10% formalsaline. After at least 48 hours, each specimen was trimmed for histopathologic orientation and processed routinely. Each specimen was embedded in paraffin wax at 90 degrees to the surface epithelium and 5 μm thick sections prepared for staining with haematoxylin and eosin, Periodic acid Schiff and Masson-trichrome at a step-serial interval of 100 μm. The sections were evaluated for tissue alteration zones induced by thermal injury and for evidence of organization of the epithelium and connective tissue. Microscopic examinations were carried out independently by two oral pathologists (Siar, Ng), who were "blinded" as to the incisional modality used and the relative age of the wounds.

RESULTS

According to the clinical observations we were able to show a complete wound closure in all of the scalpel incisions as well as the laser incisions independent on the power setting levels (Figs. 2–4).The histopathological findings presented from the superpulsed continuous wave CO_2 laser and scalpel wounds are grouped as follows.

Group 1 (Three Days Healing) (Fig. 5)

Laser wounds showed a power setting-dependent degree of thermal injury to the epithelium and underlying connective tissues with little evidence of repair at this stage. The wound site showed resolution of the epithelial surface, which was replaced by a fibrinopurulent exudate of variable thickness. In the connective tissue, there was fragmentation and disorientation of the collagen fibres and bundles with thermocoagulative changes extending to involve the submucosal minor salivary lobules. A diffuse mixed acute and chronic inflammatory cell infiltration of varying intensity was also observed. Scalpel wounds at three days were completely epithelialized and

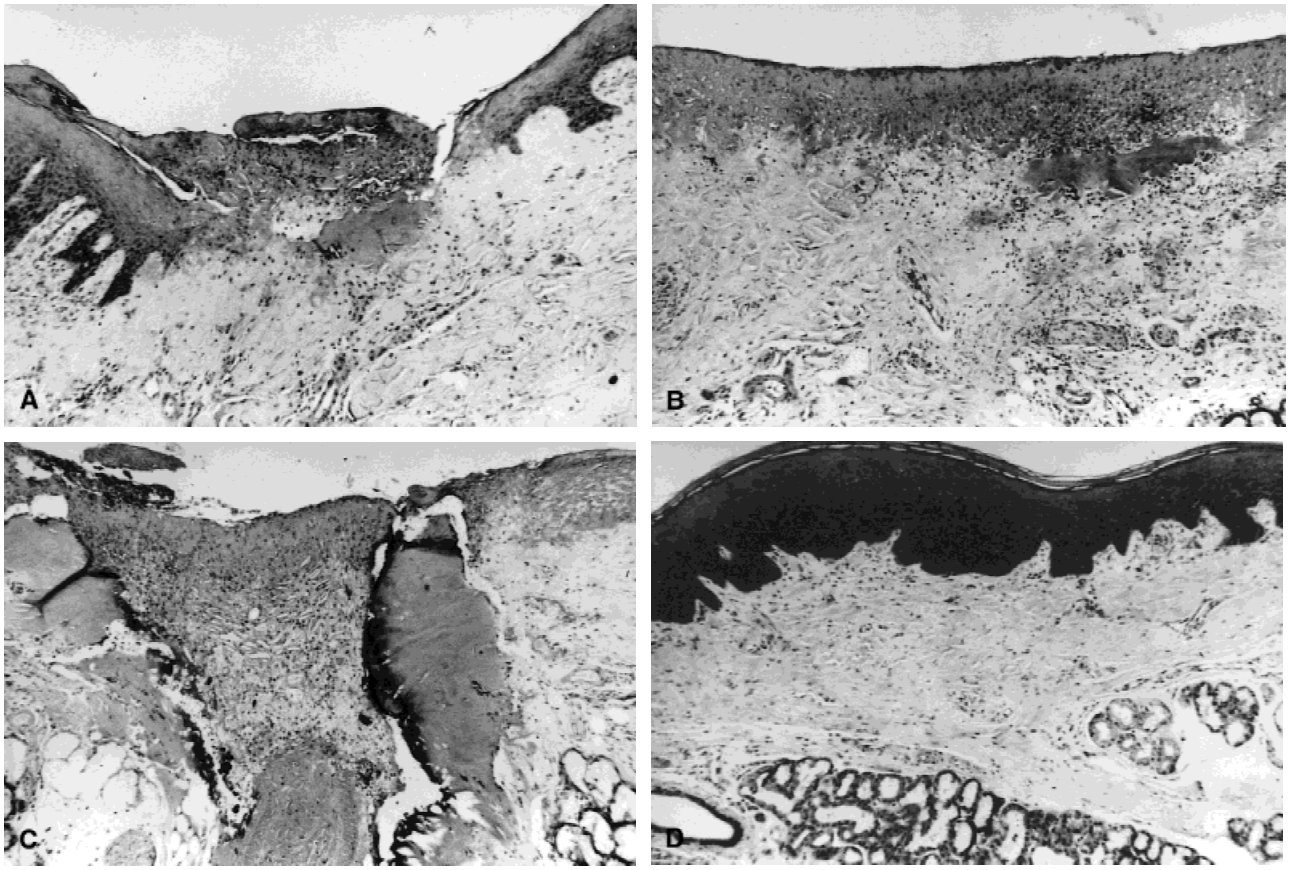

Fig. 5. Laser and conventional wounds at three days. **A:** Two weeks (2 W), **B:** 4 W, **C:** 6 W, **D:** Scalpel (H & E staining; Original magnification: 100×).

there was evidence of fibroblastic activity at the wound site. A mild mononuclear inflammatory cell infiltrate was also present.

Group 2 (One Week Healing) (Fig. 6)

Histologically, the laser and scalpel wounds were both completely epithelialized at seven days. At the laser wound site, small amounts of immature collagen fibrils interspersed between existing collagen bundles and a mild mononuclear inflammatory cell infiltrate were observed in the connective tissue. A power setting-dependent degree of thermal damage consisting of vacuolization of the subepithelial tissues remained evident. For the scalpel wound, other than a residual mononuclear inflammatory cell infiltrate in the connective tissue, there was very little evidence of wounding at this stage. The epithelial rete pegs were present only in the scalpel as well as the laser wounds with low power setting.

Group 3 (Two Weeks Healing) (Fig. 7)

Histological evaluation showed that at two weeks there was no evidence of wounding for both the scalpel and CO_2 laser wound types. A complete regeneration of the epithelium and connective tissues to normal histological constitution was consistently observed. The epithelial surface was well keratinized in all of the wounds. Epithelial rete pegs were present in the scalpel as well as laser wounds independent on the used power setting.

DISCUSSION AND CONCLUSIONS

Several studies compared the histological effects on wounds produced by CO_2 laser and conventional scalpel. With the CO_2 laser a zone of histologically-evident thermodestruction of about 60–190 μm around the laser beam and an area of reversible thermal damage for another 500 μm beyond is usually observed [1,2]. The thermal damage may range from a transient heating to protein denaturation, water evaporation, carbonization, or burning [2]. From the clinical application viewpoint, this thermal zone should ideally

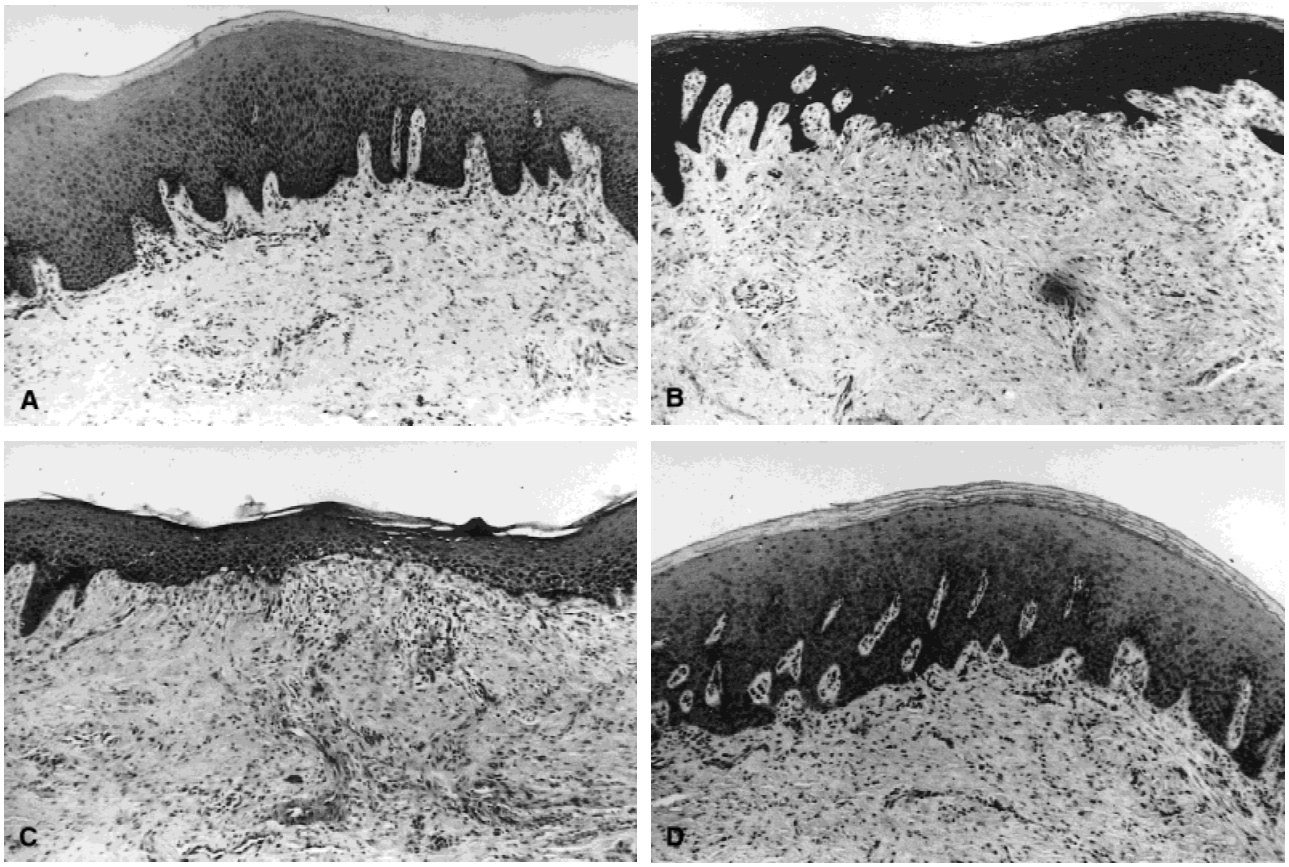

Fig. 6. Laser and conventional wounds at seven days. **A:** 2 W, **B:** 4 W, **C:** 6 W, **D:** Scalpel (H & E staining; original magnification: 100×).

be kept to a minimum as it can impede wound healing and graft take and affect its tensile strength. Wavelength of the laser, power setting (Watts), continuous/pulsed mode, pulse duration, pulse frequency, as well as exposure time are important laser parameters governing the extent of thermal injury to the tissues.

Scalpel wounds, in contrast, do not cause any thermal damage but allow extravasation of blood and lymph, causing a more marked inflammatory response with resultant swelling and formation of a scab [1,2,6].

In this study, both scalpel and superpulsed CO_2 laser wounds exhibited complete healing at 14 days with virtually no difference between the two techniques. We tried to avoid elevating the soft tissue too much during surgery and we did not use any suture material for wound closure in order to avoid incorporation of foreign bodies into the soft tissue. These findings concur well with previous studies [7–9]. However, at day 3 and day 7, the healing process for the laser wounds on the whole seems to lag behind those for scalpel wounds. Other reports too, found that laser wounds of the oral mucosa tend to show less collagen formation, little wound contraction and slower epithelial regeneration compared to conventional surgical wounds [3]. Laser wounds have also been shown to be initially weaker than scalpel incisions on skin but eventually attain similar strength. Possible explanations for the delayed re-epithelialization of laser wounds include inhibitory substances produced by necrotic tissues, physical hindrance caused by presence of eschar or heat fixation of adjacent epithelial cells [10,11]. However, a study quantifying the expression of growth factors in early wound healing showed that there was no significant differences in the expression of growth factors in the majority of time points between the CO_2 laser wounds and the scalpel wounds [12].

Evaluation of the acute inflammatory response in this study disclosed that superpulsed CO_2 laser wounds tend to be associated with more inflammatory cells compared to scalpel wounds. Hooks [13] and Yu et al. [12], who likewise en-

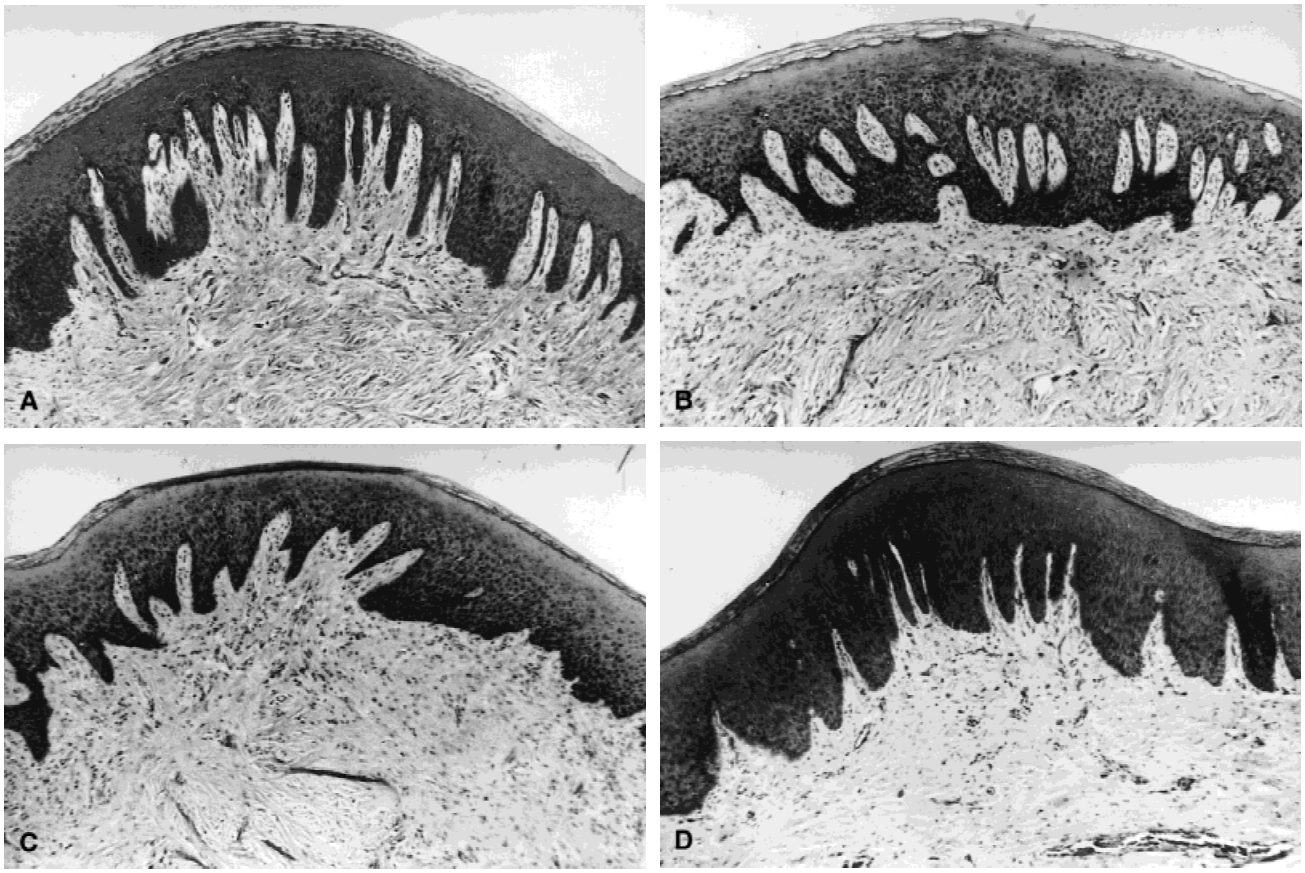

Fig. 7. Laser and conventional wounds at 14 days. **A:** 2 W, **B:** 4 W, **C:** 6 W, **D:** Scalpel (H & E staining; Original magnification: 100×).

countered these changes in their laser wound series suggested that whilst laser may reduce bacterial colonization at the time of incision, subsequent wound contamination may lead to a higher incidence of infection with resultant greater inflammatory response.

In conclusion, our preliminary results showed that superpulsed CO_2 laser tends to produce more pronounced changes (due to tissue thermal damage) with corresponding greater inflammatory reaction and delay in tissue organization only initially.

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